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Bound ^{14}C-metsulfuron-methyl residue in soils

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Abstract: Bound residue(BR) of ^{14}C-metsulfuron-methyl (^{14}C-BR) in seven kinds of soil was significantly negative-related to soil pH and positive-related to the clay content during the initial 20 d of incubation, but only was significantly negative-related to soil pH after 30 d incubation. Again, the soil pH was found to be the dominant factor affecting BR formation from ^{14}C-metsulfuron-methyl among the basic properties(soil pH, clay, OM and CEC etc.) of soil. The maximum content of ^{14}C-BR in the 7 soils accounted for 19.3% – 52.6% of applied amount. In addition, the composition of the ^{14}C-BR in fluvio marine yellow loamy(S_7) at the 90 d of incubation was identified using the coupling technique of LC-MS and isotope tracing method. The results showed that the ^{14}C-[2-amino-4-hydroxyl-6-methyl-1, 3, 5]-triazine, ^{14}C-[2-amino-4-methoxy-6-methyl-1, 3, 5]-triazine and ^{14}C-metsulfuron-methyl parent compound constituted the main components of the BR derived from ^{14}C-metsulfuron-methyl in the S_7. The relative percentage of the three compounds accounted for 41.4%, 35.8% and 19.3% of total recovery radioactivity, respectively. The results also indicated that a non-radioactive component, 2-methylformate-benzenesulfonyl-isocyanate, one of the degraded products of metsulfuron-methyl in soil, was also found to be one of the components of the BR. The parent compound in BR can well explain the phytotoxic effect on substitution crops caused by the BR derived from metsulfuron-methyl in soil.

Keywords: bound residue; composition; identification; metsulfuron-methyl; soil

Introduction

Sulfonylurea herbicides have high activity at very low application rates. Among sulfonylureas, metsulfuron-methyl[methyl 2-(4-methoxy-6-methyl-1, 3, 5-triazine-2-ylcarbamoylsulfamoyl) benzzoate] is widely used with a good selectivity, against a wide range of weeds in cereal crops, pasture and plantation crops at application rates of 4—8 g AI/hm^2 (Beyer, 1988; Brown 1990; Pons, 1998). Its target enzyme is acetolactate synthase (ALS), the enzyme which catalyses the first common reaction in the biosynthesis of branched amino acids valine, leucine and isoleucine specific to plants and microorganism (Beyer, 1988; Brown, 1990; Sweetser, 1982; Ray, 1984).

The mode of action (Beyer, 1988; Ray, 1984; Blair, 1988), degradation route (Sabadlie, 1990; 1992; 1993; Li, 1999), the fate and environmental behavior of metsulfuron-methyl (Beyer, 1988; Brown, 1990; Pons, 1998; Moyer, 1989; Walker, 1989; Hemmamda, 1994) and its sensitivity to different crops (Brown, 1990; Moyer, 1990) is well documented. Soil residues of metsulfuron-methyl have been reported to cause the same damage to rotation or substitution crops (Moyer, 1990; Kotoula, 1993; Nicholls, 1998). Recent studies have showed that the maximal amount of bound residues (BR) of metsulfuron-methyl in soil reached about 48% of the applied amount (Pons, 1998). Some groups have found that the BR derived from chlorsulfuron, 2-chloro-N-[4-methoxy-6-methyl-1, 3, 5-triazine-2-yl] amino carbonyl benzenesulfonamide, which chemical structure is similar to metsulfuron-methyl, could induce phytotoxic effect on rice (Chen, 1996; Sun, 2000). Subsequent study has confirmed that the BR derived from metsulfuron-methyl in soil could also induce phytotoxic effect on rape seedling (Ye, 2003). Guo *et al.* (Guo, 1998; 1999) have preliminarily studied the composition of the BR derived from chlorsulfuron in soil. Their results showed that the high-temperature distillation (HTD) and supercritical methanol extraction (SME) that are normal methods for extracting bound pesticide residues can destroy the original molecular structure of chlorsulfuron and its degradation products. Thus, the results based on these methods can not represent the true composition of the BR derived from chlorsulfuron in soil, and may not be applied to explain mechanism of the BR formation and the phenomena of phytotoxicity. Up to now, there are few reports on dynamics of bound residue of ^{14}C-metsulfuron-methyl in different kinds of soil. Furthermore, study on the extraction of the BR under a mild condition and identification of the composition of BR derived from ^{14}C-metsulfuron-methyl in soil is scarce.

The purpose of this paper was to investigate dynamics of BR derived from ^{14}C-metsulfuron-methyl in seven kinds of soil and its influencing factors, and to identify the composition of BR, which can also induce phytotoxic effect on rape seedling (Ye, 2003), in Fluvio marine yellow loamy(S_7) in the 90 days of incubation.

1 Materials and methods

1.1 Reagent

^{14}C-metsulfuron-methyl (Ye, 2001) (Triazine-4-^{14}C; special radioactivity, 4.5×10^4 Bq/mg; radiochemical purity and chemical purity ≥ 97%). 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine, 2-amino-4-methoxy-6-methyl-1, 3, 5-triazine as well as metsulfuron-methyl reference standard (purity > 98%, Chemical Service Co., USA). 0.1 mol/L Na_2CO_3-$NaHCO_3$ buffer (pH 10.0), 0.1 mol/L citric acid-sodium citrate buffer (pH 6.6), 0.5 mol/L KH_2PO_4, 0.5 mol/L hydride chloride, 0.5 mol/L sodium hydroxide, iso-propanol, *n*-butanol, dichloro-methane, dimethylbenzene, glycol-ether, ethanolamine, 2, 5-diphenyloxazole (PPO), 1, 4-di-[2'-(5'-phenyloxazolyl)]-benzone (POPOP) and liquid scintillation cocktail (Hisafe-3, Wallac Co., Finland) and so on. The all solvents mentioned above were analytical grade. Distilled and deionized water obtained from a Milli-Q water purification system (Millipore Co., USA). Acetic acid, acetonitrile and methanol were HPLC grade.

1.2 Apparatus

HPLC system (Waters 510, 680 and 996 etc., Waters Co., USA), solvent filtration module (Waters Co., USA), centrifugator (Universal 32, Hettich Co., Germany), decompress rotary evaporator (R-201, Institute of Shenke Machine, Shanghai, China), 4.6 mm × 250 mm C_{18} column (Supelco discovery C_{18}, Sigma-Aldrich Co., USA), C_{18} solid phase extraction column (10 mm × 150 mm, 7 μm), biological oxidizer (OX-600, Harvey Instrument Co., USA), liquid scintillation counter (Wallac 1414 LSC, Wallac Co., Finland), LC-MS (LC, Waters 2690 separation module; MS, Micromass Quattro LC, Waters Co., USA), and so on.

1.3 Incubation method for bound residues of ^{14}C-metsulfuron-methyl in soil

Seven kinds of soil used in the experiment were taken from the surface layer (0—15 cm) in the test field of Zhejiang University, and passed through a 1 mm sieve. The properties of the soil are listed in Table 1.

Four parallel samples were taken from every kind of soil. Each soil sample (240 g) was put into 500 ml flask with a rubber plug, respectively. ^{14}C-metsulfuron-methyl (3.3×10^4 Bq) dissolved in

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methanol was added into each soil sample, and stirred in a vent hood. After all methanol were removed, distilled water was added into the soils until the water content was adjusted to 60% of soil water holding capacity(WHC), continue to stir the soil until it was uniform. In order to prevent ^{14}C-CO_2 escaping from the samples, a 20 ml-vial filling with 10 ml 0.5 mol/L NaOH was hung under the rubber plug. The flasks were maintained at 25 (± 1)℃ in the dark. During the course of incubation, the water content was kept constant by adding distilled water every day. At the 1, 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 d of incubation, a part of the soil corresponding to 20 g air-dried soils was sampled, and then extracted by methanol in a vibrator for five times continuously until all the extractable residues of ^{14}C-metsulfuron- methyl in soil was thoroughly removed. In each step mentioned above, the spiked soil sample was extracted for 2 h and subjected to centrifugate (4000 r/min) for 15 min. The efficiency of the continuous methanol-extraction for extractable residues of ^{14}C-metsulfuron-methyl in soil is higher than that of Soxhlet extraction method(24 h). The residual soil after the continuous methanol extraction was termed as ^{14}C-BR sample in this study.

Table 1 Properties of the soil samples

Soil No.	Types of soil	pH (H_2O)	OM, g/kg	CEC, cmol/kg	Clay, %	Silt, %	Sand, %
S_1	Paddy field on quaternary red soil	5.36	15.7	13.7	39.0	41.4	19.9
S_2	Paddy field on red sandstone soil	5.61	11.3	12.3	17.2	7.4	75.4
S_3	Paddy field on redeposit of purple mudstone soil	5.82	20.3	15.9	22.1	50.3	27.6
S_4	Coasial saline soil	9.04	9.50	7.1	24.3	71.1	4.6
S_5	Blue clayey paddy soil	6.20	40.6	25.1	35.3	60.6	4.1
S_6	Silt clayey yellow mottled paddy soil	6.22	31.5	28.5	38.0	57.0	5.0
S_7	Fluvio marine yellow loamy	7.06	30.5	16.3	8.0	71.3	20.8

1.4 Total ^{14}C-BR content analysis

^{14}C-BR sample(1 g) was combusted for 4 min in the biological oxidizer, the evolved ^{14}C-CO_2 was absorbed in 15 ml liquid scintillation cocktail(10 g PPO + 0.5 g POPOP + 500 ml dimethylbenzene + 175 ml ethanolamine + 325 ml glycol-ether) for radioactivity measurement by liquid scintillation counter (LSC), each analytical procedure was replicated for four times. The total radioactivity (A_T) in 10 g ^{14}C-BR sample was calculated.

1.5 Extraction and purification method of bound residues derived from ^{14}C-metsulfuron-methyl in soil

About 10 g ^{14}C-BR sample at the 90 d of incubation was extracted and purified following the procedure described by Ye *et al*. (Ye, 2004). All the extracting solutions were mixed and concentrated under decompressed condition at 60℃ until all the acetonitrile was removed, and then the radioactivity of the extracting part (A_E) was determined. The radioactivity (A_r) of residues in soil was also determined by LSC after the residual soil was combusted. The efficiency of the extracting procedure was calculated with the formula $(A_T-A_r)/A_T$. Subsequently, the pH of the extracting solution was adjusted to 3.3 with 0.5 mol/L HCl, and extracted by dichloromethane(V/V = 1:1) for four times. The organic phase was concentrated to 50 ml by decompress rotary evaporator, the water phase was continued to be extracted by the mixed solution of iso-propanol and ethyl acetate(V/V = 1:1) for four times. Combined the organic phase and vaporized it under decompressed condition at 60℃. Solution of 0.1 mol/L Na_2HCO_3-Na_2CO_3 was added into the concentrated organic phase, shaked it vigorously and transferred it to a distributary funnel in order to separate the organic phase and the water phase. This process was triplicated. The water phase was filtered through a 0.45 μm ultra-filtrate membrane and adjusted to pH = 3.3, the filtrate was passed through a C_{18} pretreatment column(10 mm × 150 mm, 7 μm). The component with radioactivity that can not be hold on the pretreatment column was collected(marked as fraction 1), while the retained component(marked as fraction 2) in the pretreatment column was eluted by ethyl acetate. The fraction 2 was concentrated in a condense tube until all the solvent was removed. The fraction 1 in the water phase was again extracted by chloroform (V/V = 1:1) for three times. Discarded the chloroform, then the water phase was extracted by a mixed solution of iso-propanol and ethyl acetate with volume ratio of 1:1. Repeated three times and mixed the organic phase, which was condensed to a small volume subsequently. Combined it with the fraction 2 and condensed to 2 ml. The radioactivity (A_R) of the concentrated solution was determined so as to calculate the recovery rate (A_R/A_E) over the process of the purification. The remain solution of the sample was kept in 4℃.

1.6 Identification of the composition of BR derived from ^{14}C-metsulfuron-methyl in soil (S_7) with LC-MS

The composition of ^{14}C-BR in the S_7 at the 90 d of incubation was identified with LC-MS. The analysis condition for LC-MS was as follows: a 4.6 mm × 250 mm C_{18} column was used as the separation column, the flow rate of mobile phase was 1 ml/min in a gradient elution mode (minutes/% A: 0—2/90, 2—12/65, 12—20/65, 20—28/20, 28—30/20, 30—35/90), solution A was a mixture of water and 0.1% glacial acetic acid (1 + 1 by volume), solution B was a mixture of acetonitrile and 0.1% glacial acetic acid(1 + 1 by volume). The UV detector used was a photodiode array detector. The mass spectrometer was Micromass Quattro LC. Electrospray positive ion (ES^+) and negative ion (ES^-) full scan (100—600 amu) was performed respectively, meanwhile, the selected ion with 127, 141 and 382 of m/z for ES^+ scan was also detected. The injection volume of the S_7-90d-BR sample was 40 μl.

1.7 Radioactive determination of several fractions eluted from HPLC

Under the same chromatographic condition, 200 μl(40 × 5) of the S_7-90d-BR sample was injected into the HPLC system. The fractions eluted from HPLC column with the retention time ranging in 2.5—4.1, 4.2—5.0, 5.1—6.5, 6.5—8.1, 8.1—9.5, 9.5—10.3, 10.3—11.7, 11.7—13.2, 13.2—14.3, 14.4—15.4, 15.5—16.7, 16.8—18.4, 18.4—20.0, 20.0—22.0 and 22.0—24.0 min were collected and concentrated, respectively. 10 ml Hisafe-3 scintillation liquid was added into every fraction mentioned above and its radioactivity was respectively detected by LSC. Each of detection was triplicated. After while, 10 ml Hisafe-3 scintillation liquid was added into another 200 μl S_7-90d-BR sample, and the radioactivity in 200 μl BR sample was also determined for the calculation of the radioactive percentage in each fraction.

2 Results and discussion

2.1 Dynamics of BR derived from ^{14}C-metsulfuron-methyl in different kinds of soil and its influencing factors

The change of BR derived from ^{14}C-metsulfuron-methyl in different kinds of soil is shown in Fig.1. There was no significant difference of the ^{14}C-BR content derived from ^{14}C-metsulfuron-methyl in S_2, S_3, S_5 and S_6 during the initial 20 d of incubation, but there was obvious difference of the ^{14}C-BR content among the four soils after 20 d of incubation. During 60 d of incubation, in S_1, S_4 and S_7, the changed trend of the ^{14}C-BR content with time was evidently different compared with the above-mentioned four soils. The result also indicated that after 60 d of incubation, the ^{14}C-BR content in S_1—S_7 reached the maximum, accounting for 48.5%, 46.5%, 52.6%, 19.3%, 49.7%, 42.0% and 46.5% of applied amount, respectively. Except for S_4, the content of ^{14}C-BR in other 6 kinds of soil was about half of the applied amount. Pons *et al*. (Pons, 1998) also demonstrated that after 98 d of incubation, the maximum content of ^{14}C-BR in acerbic soil was about 48% of applied amount. Thus, it could be concluded that the bound ^{14}C-metsulfuron-methyl residue in soil was remarkable.

The results of partial correlation analysis with Statistical Package for the Social Science (SPSS8.0)(Table 2) indicated that BR of ^{14}C-metsulfuron-methyl in the soil was significantly negative-related to soil pH and positive-related to the clay content(showed in expression 2) during the initial 20 days of incubation, considering soil pH, clay, OM and CEC and so on. Therefore, ^{14}C-BR was much more easily formed in acerbic and high-clay soil during that phase. However, after 30 d, ^{14}C-BR was only negative-related to soil pH in the 7 soils, significantly. Expression 2 could be simplified as expression 1, thus it could be

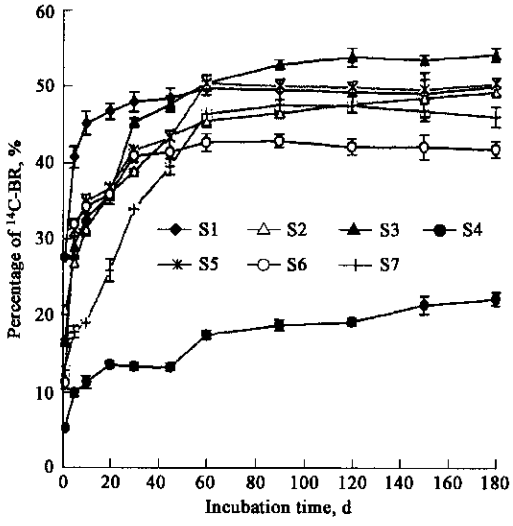

Fig.1 Dynamics of bound ^{14}C-metsulfuron-methyl residue in soils

concluded that soil pH was the crucial factor among soil pH, clay, OM and CEC etc., which influenced ^{14}C-BR in soil during the whole incubation.

Table 2 The relationship between soil properties and ^{14}C-BR content in soil

Incubation time, d	Expression	Correlation coefficient	F	P
5	$BR_5 = 54.3\text{-}6.20pH + 0.459clay^*$ or	0.995	183.5	0.000
	$BR_5 = 71.0\text{-}6.93pH^{**}$	0.846	12.6	0.016
10	$BR_{10} = 61.5\text{-}6.99pH + 0.492clay^*$ or	0.991	106.5	0.000
	$BR_{10} = 79.4\text{-}7.78pH^{**}$	0.853	13.3	0.015
20	$BR_{20} = 71.2\text{-}7.34pH + 0.326clay^*$ or	0.993	133.4	0.000
	$BR_{20} = 83.1\text{-}7.86pH^{**}$	0.926	29.9	0.003
30	$BR_{30} = 95.0\text{-}9.03pH^{**}$	0.964	66.1	0.000
45	$BR_{45} = 101.1\text{-}9.64pH^{**}$	0.985	163.6	0.000
60	$BR_{60} = 100.6\text{-}9.01pH^{**}$	0.944	41.0	0.001
90	$BR_{90} = 100.3\text{-}8.85pH^{**}$	0.939	37.2	0.002
120	$BR_{120} = 100.4\text{-}8.84pH^{**}$	0.937	36.0	0.002
150	$BR_{150} = 96.5\text{-}8.22pH^{**}$	0.942	39.2	0.002
180	$BR_{180} = 96.8\text{-}8.23pH^{**}$	0.943	40.0	0.001

Notes: * Expression 1; ** expression 2

2.2 The extraction efficiency and the recovery rate of ^{14}C-BR in the overall processes of extraction and preparation

The extraction efficiency of the ^{14}C-BR derived from ^{14}C-metsulfuron-methyl in the 7 soils at the 90 d of incubation and the recovery rate of ^{14}C-substances in the overall processes of extraction and preparation are listed in Table 3. It was found that the extraction efficiency of the ^{14}C-BR in different kinds of soil samples was ranged from 79.5% to 83.8%. The results also showed that there were no significant differences in extraction efficiency among the different kinds of soil samples.

Table 3 Extraction efficiency of the ^{14}C-BR and the recovery rate of the overall processes of extraction and preparation ($n = 4$)

BR sample No.	Extraction efficiency, %	Recovery rate of the overall processes, %
S_1-90-BR	79.5 ± 2.0	63.9 ± 3.3
S_2-90-BR	82.4 ± 1.4	66.2 ± 3.0
S_3-90-BR	83.8 ± 1.7	67.4 ± 3.1
S_4-90-BR	82.8 ± 2.0	66.6 ± 3.3
S_5-90-BR	83.6 ± 1.2	67.2 ± 2.9
S_6-90-BR	83.5 ± 0.8	67.1 ± 2.7
S_7-90-BR	80.8 ± 1.2	65.0 ± 2.9

The pretreatment of the BR sample was a very complicated and difficult process. The extracted solution of the BR soil sample was in the color of dark-brown and contained high content of impurity, along with high content of polar degradation products of ^{14}C-metsulfuron-methyl. It was finally found that the polar components of the ^{14}C-BR in aqueous phase could be effectively extracted by the mixed solution of iso-propanol and ethyl acetate(V/V = 1:1), the extraction recovery rate could reach 90.5%. It can be seen from Table 2, the recovery rate of ^{14}C-BR in the overall processes of extraction and preparation reached 63.9%—67.4% even though the pretreatment of the ^{14}C-BR sample was a complicated and multistep process.

2.3 Identification of composition of BR derived from ^{14}C-metsulfuron-methyl in soil (S_7)

The component with radioactivity must be derived from the ^{14}C-metsulfuron-methyl molecular. Thus, the LC-MS determination process was chiefly focused on the ^{14}C-labeled triazine ring so as to identify the BR composition derived from ^{14}C-metsulfuron-methyl in S_7 conveniently. The total ion chromatogram(TIC) in the mode of electrospray positive ion full scan(100—600 amu) is shown in Fig.2. Each peak fraction was collected under the same chromatographic condition and its radioactivity was detected, the results are listed in Table 4.

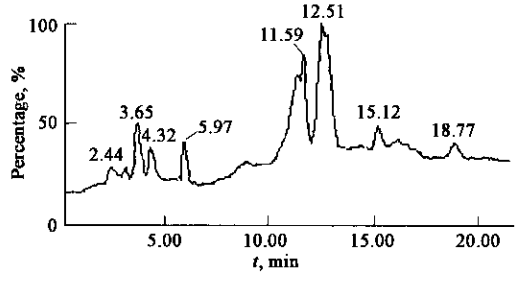

Fig.2 LC-MS total ion chromatogram(ES^+) of ^{14}C-BR in soil

Table 4 The retention time of the BR component with radioactivity and their relative percentage accounted in total recovery radioactivity

Retention time, min	The relative percentage accounted in total recovery radioactivity, %
2.5—4.1	41.4
5.0—6.5	35.8
18.0—20.0	19.3

Table 4 shows that the three components with the retention time of 2.5—4.1, 5.0—6.5 and 18.0—20.0 min(marked as component Ⅰ, Ⅱ, Ⅲ, respectively) had the radioactivity, therefore, the structure of these three components was identified in details, respectively.

It can be seen from Table 4, the component Ⅰ with retention time of 2.5—4.1 min had radioactivity. Its relative percentage accounted in total recovery radioactivity reached 41.4%. The results of the selected ion(m/z = 127, ES^+) chromatogram and mass spectrum demonstrated that the molecular weight of components Ⅰ was 126(Fig.3 and Fig.4). The reasonable explanation for the structure of component Ⅰ with molecular weight of 126 should be ^{14}C-[2-amino-4-hydroxyl-6-methyl-1, 3,5]-triazine since the component Ⅰ had radioactivity and derived from the triazine ring.

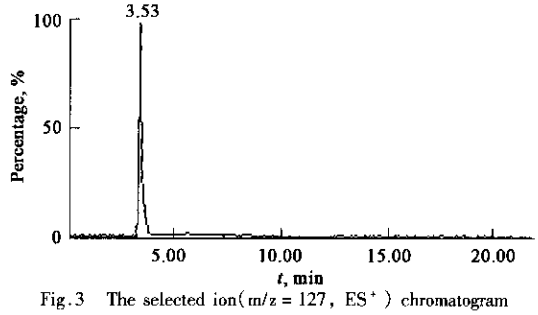

Fig.3 The selected ion(m/z = 127, ES^+) chromatogram

From Table 4, it was found that the component Ⅱ with retention time of 5.0—6.5 min also had the radioactivity. The relative percentage of this compound accounted for 35.8% of total recovery radioactivity. The selected ion (m/z = 141, electrospray positive ion scan)

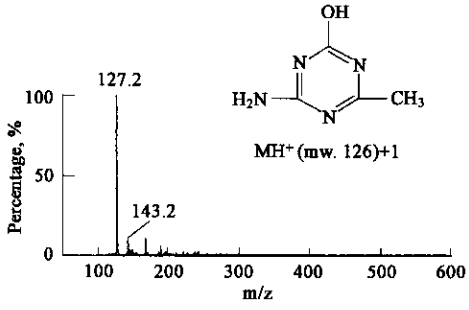

Fig.4 Mass spectrum(ES⁺) of the compound with the retention time of 3.6 min

chromatogram showed that the compound with the retention time of 6.1 min might had a fragment or molecular with m/z = 141 (Fig.5). The mass spectrum(electrospray positive ion full-scan) demonstrated that the molecular weight of the compound with the retention time of 6.0 min was 140, and the reasonable structure should be ^{14}C-[2-amino-4-methoxy-6-methyl-1,3,5]-triazine since the compound had the radioactivity and must be related to the triazine ring(Fig.6).

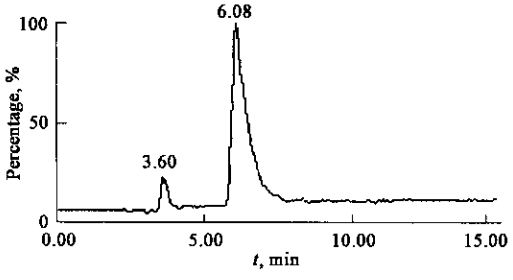

Fig.5 The selected ion(m/z = 141, ES⁺) chromatogram

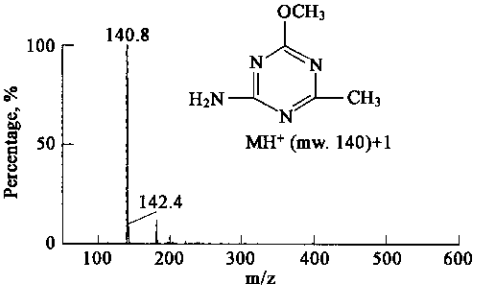

Fig.6 Mass spectrum(ES⁺) of the compound with the retention time of 6.0 min

The mass spectrum (electrospray positive ion full-scan) of the compound with the retention time of 15.1 min is illustrated in Fig.7. It was shown that there was a fragment with m/z = 224, it might be adduct of 2-carboxylic-benzenesulfonamide with sodium ion. The molecular weight of this component should be 241 (MH^+, mw. 241 + 1). Consequently, the reasonable structure of the compound should be 2-methylformate-benzenesulfonyl-isocyanate. Sabadie had also reported that 2-methylformate-benzenesulfonyl-isocyanate was one of the degradation products derived from metsulfuron-methyl(Sabadie, 1992; 1993).

The selected ion (m/z = 382, electrospray positive ion scan) chromatogram showed that the compound Ⅲ with the retention time of 18.8 min might had a fragment or molecular with m/z = 382(data not shown). The mass spectrum(electrospray positive ion and negative ion full-scan) also demonstrated that there were three fragments with the m/z 141(ES^+), m/z 167(ES^+) and m/z 139(ES^-, data not shown). The deductive structures of the three fragments(Reiser, 1991) are shown in Fig.8. The molecular weight of the compound with the retention time of 18.8 minutes was 381. Based on all the above information, it could be concluded that the component Ⅲ was the parent compound (^{14}C-metsulfuron-methyl).

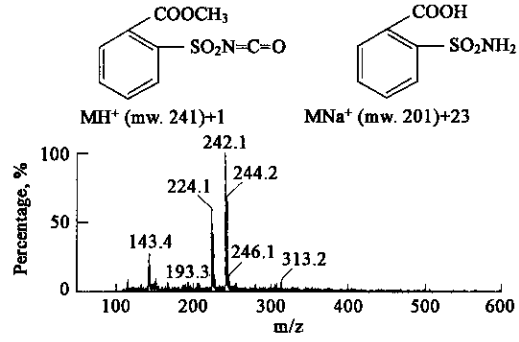

Fig.7 Mass spectrum (ES⁺) of the compound with the retention time of 15.1 min

For further verifying the validity of the conclusion, the chromatographic retention time of reference standards (2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine, 2-amino-4-methoxy-6-methyl-1, 3, 5-triazine was well as metsulfuron-methyl) was compared with that of component Ⅰ, Ⅱ and Ⅲ, the results were confirmed that their retention times were in accord with that of the relevant standart. Therefore, the ultimate conclusion that the ^{14}C-metsulfuron-methyl parent compound existed in the bound residues could be reached. This conclusion is very important to explain the phytotoxicity induced by the BR of metsulfuron-methyl in soil.

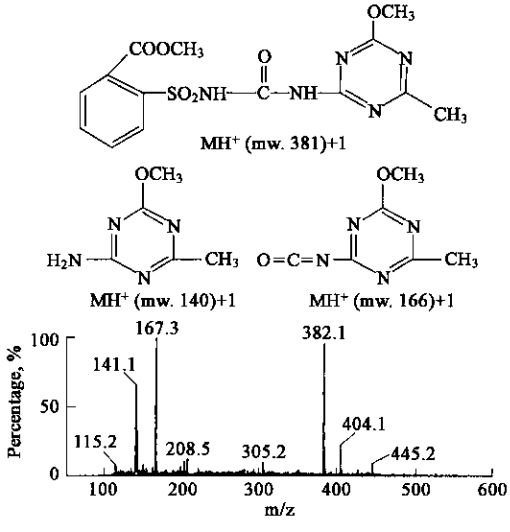

Fig.8 Mass spectum (ES⁺) of the compound with the retention time of 18.8 min

The final results of the BR composition derived from ^{14}C-metsulfuron-methyl in soil are listed in Table 5. From Table 5, it could be concluded that the ^{14}C-[2-amino-4-hydroxyl-6-methyl-1,3,5]-triazine, ^{14}C-[2-amino-4-methoxy-6-methyl-1,3,5]-triazine, ^{14}C-metsulfuron-methyl parent compound and 2-methylformate-benzenesulfonyl-isocyanate constituted the main composition of the ^{14}C-BR. Sabadie (Sabadie, 1990; 1992; 1993) and Li *et al*.(Li, 1999) had verified that the 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine and 2-amino-4-methoxy-6-methyl-1, 3, 5-triazine were the main degradation products of metsulfuron-methyl.

Table 5 The BR composition derived from ^{14}C-metsulfuron-methyl in soil and the relative percentage accounted in total recovery radioactivity

Name of component in the BR	MW.	The relative percentage accounted in total recovery radioactivity, %
^{14}C-2-amino-4-hydroxyl-6-methyl-1,3,5-triazine	126	41.4
^{14}C-2-amino-4-methoxy-6-methyl-1, 3, 5-triazine	140	35.8
^{14}C-metsulfuron-methyl parent compound	381	19.3
2-methylformate-benzenesulfonyl-isocyanate	241	/

2.4 The probable mechanism of ^{14}C-BR formation in soil

Ion exchange may be the main interaction mode in the formation of ^{14}C-BR derived from ^{14}C-metsulfuron-methyl in soil. It is known that the nitrogen atom in s-triazine can be easily protonated and the protonated triazine ring can be strongly bound to humic substance in soil (Senesi, 1980). Since ^{14}C-[2-amino-4-hydroxyl-6-methyl-1, 3, 5]-triazine and ^{14}C-[2-amino-4-methoxy-6-methyl-1, 3, 5]-triazine comprise the triazine ring, both of them might be protonated and bound to fuvic acid and humic acid through ion exchanging force. Our results showed the ^{14}C-BR content increased with the decreasing of soil pH. Moreover, soil pH was the crucial factor which influenced formation of bound ^{14}C-metsulfuron-methyl residue in soil during the whole incubation among soil pH, clay, OM and CEC etc. With the mechanism above-mentioned, this result can be well explained. The more acid the soil was, the more proponated 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine and 2-amino-4-methoxy-6-methyl-1, 3, 5-triazine would present in soil and bind to soil matrix.

Metsulfuron-methyl parent compound might bind to the soil matrix through a mixed interaction mode. It is well documented that the interaction mode between pesticide and soil is dependent on the chemical structure of the herbicide as well as the properties of the soil. The types of mechanism involved in binding process include ionic, hydrogen and covalent bonding, charge-transfer or electron donor-acceptor mechanism, Van der Waal forces, ligand exchange, hydrophobic bonding or partitioning (Baily, 1970; Khan, 1982; Senesi, 1992; Pignatello, 1996; Gevao, 2000). The configuration, electronic structure and physico-chemical property of sulfonylurea pesticides have been well studied from the point view of quantum chemistry (Yan, 1998). The heterocyclic ring in metsulfuron-methyl was electron-deficient and may interact with electron-rich sites on soil through electron transfer interactions. The benzene ring in metsulfuron-methyl may interact with soil organic matter via hydrophobic interactions, such as π-π charge transfer. The electron-drawing substituent on the benzene ring and the sulfur atom in the bridge of the molecule may interact with electron acceptors in soil organic matter through electrostatic forces. Moreover, the oxygen in the sulfonyl group may interact with electron acceptors in soil through hydrogen bonding. It is well understood that a given chemical might undergo bond interaction via several mechanisms simultaneously (Caiderbank, 1989). Multiple mechanisms may be also expected for the binding between metsulfuron-methyl parent compound and soil matrix.

The metsulfuron-methyl parent compound can be also found in the form of BR after 90 d of incubation, which can be well explained the phytotoxic effect on substitution crops caused by the BR derived from metsulfuron-methyl in soil.

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